Microbiota profiling in gastrointestinal health and disease

9

Valorisation

VALORISATION

Relevance for society and economy

Chronic disorders, such as obesity, liver cirrhosis and inflammatory bowel disease (IBD; primarily including Crohn's disease and ulcerative colitis), account for nearly two-thirds of deaths world-wide and pose a considerable financial burden to health care and society [1]. For obesity, costs are estimated at $190 billion in 2005 in the USA and expected to increase by $48-66 billion/year in 2030 [2]. For liver cirrhosis and chronic liver diseases costs are estimated at $2.5 billion USD/year in USA [3], while for ulcerative colitis the direct costs are estimated at $4 billion/year in the USA [4] and for Crohn's disease costs are estimated at $30 billion annually in the USA and Europe [5]. Furthermore, these disorders are expected to increase in the years to come.

Obesity rates are currently rising worldwide, especially in low- and middle income countries [6]. The number of children who are overweight or obese has nearly doubled from 5.4 million in 1990 to 10.6 million in 2014. There are more obese than underweight individuals in almost every region of the world (except for parts of sub-Saharan Africa and Asia) and comorbidities (*e.g.* cardiovascular diseases and diabetes) are responsible for more than 2.5 million deaths per year world-wide [7]. A major goal of obesity treatment is weight loss to limit or prevent complication, which can be achieved by lifestyle changes (although relatively ineffective with regard to long term weight loss) and bariatric surgery [6].

The prevalence of liver cirrhosis is also expected to rise worldwide. In 2010, it was estimated that liver cirrhosis is the cause of one million deaths worldwide (about 2% of all deaths) [8]. However, estimations of the exact prevalence are difficult, especially as compensated cirrhosis, the first stage of liver cirrhosis without complications, may not always be diagnosed [9,10]. Fatty liver disease, viral infections (hepatitis) and alcohol abuse are well-known causes of liver cirrhosis. The obesity epidemic discussed earlier is associated with the metabolic syndrome and non-alcoholic fatty liver disease (NAFLD), which are conditions that also contribute to the expected rise of patients progressing into liver cirrhosis in the future.

Crohn's disease and ulcerative colitis, also known as IBD, are generally considered to be 'Western' diseases, but the incidence is emerging worldwide in line with westernization [11]. This contributes to a high prevalence of IBD, which is also affected by the chronicity of the disease, young age of onset and low mortality. The health care system needs to be prepared for the rise of IBD patients in general, but also for the increase of the ageing IBD population with its more complex comorbidities [11,12]. Over the last decade, the treatment goal shifted from improvement of clinical symptoms towards achieving mucosal healing and prevention of long term complications, facilitated by the introduction of biological agents (*i.e.* anti-TNF) [13]. Since the demand for these expensive drugs is emerging, the high costs of these new treatments,

predicting treatment response and selecting the right treatment for the right person are an ongoing topics of debate.

Next to direct health care costs, the above mentioned diseases also have significant impact on the quality of life of the patients and indirect costs. Considering the current state, further research to decrease disease burden and improve current treatment options are warranted. On one hand this involves further insight in the pathophysiology and on the other hand non-invasive markers for early diagnosis and monitoring of disease course are urgently needed. All of the three mentioned disorders can be characterized by a complex multifactorial pathophysiology, including *e.g.* changes in the intestinal microbiota composition. Further studies on these microbial changes are needed to explore whether the microbiota can be used as a diagnostic or disease progression marker and to gain further insight in the mechanisms involved in onset and progression of these disorders.

Target groups

In this thesis, we focused on the microbiota of three different disorders, namely obesity, liver cirrhosis and Crohn's disease. Not only basic scientist and clinicians, but also companies that are developing novel diagnostic methods or intervention therapies based on the microbiota profiles may benefit from the results presented in this thesis. Furthermore, the results of the sampling and storage study in the second chapter are of relevance for scientists and clinicians to determine which sampling and storage methods should be used when setting up microbiota studies. The high direct (medical) and indirect (loss of work productivity) costs associated with the respective disorders also affects the general population. The results of this thesis could benefit the general population since the results might accelerate the development of novel diagnostic methods and treatments that are able to mitigate the financial burden of these disorders.

Innovations and activities

Since the microbiota plays an important role in health and disease, it should not come as a surprise that microbial changes have been associated with a plethora of various diseases and even different disease stages. This discovery has drawn attention of various scientists and companies to utilize the microbiota for diagnostic purposes or to develop microbiota targeted treatments and resulted in increasing effort in the microbiota research.

In this thesis, we have demonstrated that the microbiota composition in patients with CD, obesity or liver cirrhosis is different from controls. In general, a lower microbial diversity was found between patients with CD, liver cirrhosis and individuals with obesity as compared to controls. Although a loss of microbial diversity is often found in diseased individuals, a general conclusion or statement about the role of microbial diversity in health and disease can not be made since different types of host-microbe interactions are involved in the pathophysiology

of different diseases [14]. A higher abundance of SCFA producing genera (*Eubacterium, Dorea, Ruminococcus* and *Blautia*) was found in obese as compared to lean individuals. This observation supports the concept of an "obese" microbiota that might stimulate obesity development via increasing the energy harvest from diet.

Furthermore, we showed that not a single bacterial group or species, but a set of 50 bacterial taxa was found which can discriminate between active CD and CD in remission. Disease severity was found to have a pronounced impact on the microbiota composition in patients with liver cirrhosis. These data show the potential to use fecal microbiota data for disease monitoring. These findings need to be confirmed in further (prospective) studies, including the longitudinal, follow-up of patients.

Since the current state of the art technology based on next generation sequencing is not easy to implement in daily clinical practice, we applied a microbiota profiling technique (IS-pro) in the liver cirrhosis study. We demonstrated that IS-pro is a promising tool for noninvasive monitoring in clinical care and can be used for various microbiota-associated diseases.

In addition to microbiota-based disease monitoring or diagnostics, the microbiota may also have potential to be used to predict treatment responses. This is especially valuable for treatments that are known to be effective only in smaller percentages or subgroups of patient populations. An example is the substantial rate of non-responders in anti-TNF treatment in IBD [15]. Although this was not investigated in the current thesis, the analysis method that we applied (Random Forest) can also be used to identify microbial taxa that are able to predict treatment response and thereby may help future initiatives.

Clinicians, researchers and companies/industry are currently investigating the possibility to develop microbiota-based treatments. Although theoretically promising, evidence-based microbiota targeted therapeutics are still limited at this moment. Products containing beneficial microbes such as *Lactobacillus, Bifidobacterium* and *Saccharomyces* are currently available to combat travelers' or antibiotic-associated diarrhea. However, interventions for intestinal diseases are not readily available or implemented in standard health care, in part due to limited methodological quality of the studies and too generalized (non-targeted) approaches. Further insight in microbial perturbations and how microbiota profile changes contribute to disease development or progression, will aid in designing more targeted interventions. One of the few microbiota interventions available for intestinal disease is fecal transplantation, which 'replaces' the intestinal microbiota of diseased individuals with the fecal microbiota of a healthy individual. This method has been proven to be effective against (recurrent) *Clostridium difficile* infections, but potential risks (*e.g.* transfer of pathogens or antibiotic resistance genes) have to be taken into account. Safety issues in fecal transplantation, especially on long term, need to be addressed.

The bacterial taxa that we identified to be associated with diseases and disease states in this thesis are valuable in this respect and can also provide new insights regarding the role of the microbiota in disease. For example, *Bacteroides fragilis* (amongst others) was found to be associated with exacerbations in CD. A subgroup of *B. fragilis* is able to produce toxins which can affect intestinal permeability and aggravate intestinal inflammation. On the other hand, we also found *Faecalibacterium prausnitzii* (amongst others) to be associated with remission of CD. *F. prausnitzii* is known to be a major butyrate producer. Butyrate is a short chain fatty acid (SCFA) that is a major energy source for epithelial cells and plays an important role in maintaining the intestinal barrier integrity and has anti-inflammatory effects on the host [16]. *F. prausnitzii* is also able to dampen inflammation for example by producing metabolites that inhibits NFκB activation and IL-8 secretion [17]. Successful microbiota targeted interventions can act by promoting the growth of microbes associated with remission or combat microbes associated with active inflammation by using microbial compounds, antagonists and pre- or probiotics.

Perspectives

Although the concepts on the intestinal microbiota as non-invasive disease markers and on microbial intervention strategies are promising, the successful translation of such (basic) microbial research into clinical applications remains challenging. Therefore, further validation, standardization and tight collaborations between clinicians, scientists and industry is needed.

Although the microbiota obtained from intestinal biopsies are considered to be more valuable for examining the role of the microbiota in several disease states, due to the proximity of the microbiota to the host, these samples are not suitable for routine monitoring. Repeated samples are necessary and obtaining biopsies via endoscopy requires invasive and expensive procedures.

Feces or exhaled breath are rather easy to obtain and can give a valuable reflection of the microbiota composition and/or its activity. Immediate freezing at -80 °C or direct DNA isolation after defecation is the best method to investigate the microbiota composition, but this is often not possible when including patients from clinical or outpatient settings. The choice of sampling and storage methods is therefore crucial in the realization of reliable microbiota-based monitoring or diagnosing tools. In addition, costs for sampling and storage methods are a decisive factor in the realization of novel microbiota-based tools in the clinic. Various companies have developed patient friendly sampling and storage methods/kits that guarantees to keep the microbial integrity intact, even after several days at room temperature, making it possible to send the samples via mail. However, it is important that these methods/kits are affordable. Since we found that the fecal microbiota composition can be kept stable up to 24 hours at room temperature, auto-collection of fecal samples at the patients' home is possible in case the samples are delivered at the hospital or diagnostics department within 24 hours. This is especially convenient when the moment of sampling coincide with doctors appointments (as in the case of the IBD-South Limburg cohort). In contrast to feces, exhaled air is easy to collect

at any time and at any place in any patient. Previous studies from our group showed that analyses of volatile organic compounds (VOCs) in exhaled air resulted in sensitive and specific VOC profiles for the diagnosis of IBD, irritable bowel syndrome and liver cirrhosis [18–20]. In the current thesis, we found a correlation between VOCs and microbial taxa specific for disease status (remission or exacerbation). This may be valuable for elucidating the functionality of the microbiota and its role in Crohn's disease. Further studies are needed to investigate whether combining microbiota analyses and metabolites in exhaled air can increase their diagnostic accuracy as a disease (activity) marker.

Developing microbiota-based treatments often involves delivering specific strains, microbial products or other molecules to the intestine via the oral route. Targeted delivery of these compounds directly in the intestine, thereby reducing deleterious effects for *e.g.* gastric acid, bile and pancreatic enzymes, may increase the efficacy. Delivery platforms (*e.g.* enteric coatings) and synthetic biology can be helpful. The latter is a relatively new and upcoming branch of biology that involves designing or redesigning (biological) compounds and (biological) systems for useful purposes. Bacterial strains can be modified to result in an increased production of beneficial metabolites or may be engineered in such a way to have an advantage over other microbes (*e.g.* competing for nutrients or adhesion sites). Novel interventions need to be tested prior to implementation in the clinic. This is often done in animal models, but it is important to note the intrinsic differences between these and humans. *In vitro* intestinal models such as the TIM system can be of additional value to test delivery systems, since they closely mimic the human gastrointestinal tract [21,22].

In summary, this thesis contributes to the ongoing debate whether and how microbiota can be used as a diagnostic tool, marker for disease progression and predictor for response to therapeutic interventions. We have shown that for the development of microbiota-based markers, it is better to search for a set or combination of multiple members of the microbiota rather than to focus on only a few members. Metabolites produced by the microbiota are also able to reflect or even influence host' health and should be taken into account. In addition, we provided information for selecting the correct sampling and storage method applicable in the clinical setting, which is patient-friendly and maintains the microbiota composition. Microbiota research is fast-pacing and we are currently moving from describing the microbiota composition to elucidating their functions and dynamics. There is a need for mechanistic studies to elucidate interactions between members of the microbiota together and their host. This knowledge will enable the development of new interventions to manipulate the microbiota with the intention to improve human health. It should be taken into account that efforts to manipulate the interactions between the host and microbiota may result in unforeseen adverse outcomes (*e.g.* potential pathogenicity of commensal microbes). Therefore, further interdisciplinary investigations will lead to better understanding of the microbiota-host interactions and will contribute to safe microbiota-based interventions against a range of disorders.

REFERENCES

1. Bauer UE, Briss PA, Goodman RA, Bowman BA. Prevention of chronic disease in the 21st century: elimination of the leading preventable causes of premature death and disability in the USA. Lancet. 2014;384: 45–52. doi:10.1016/S0140-6736(14)60648-6
2. Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M. Health and economic burden of the projected obesity trends in the USA and the UK. Lancet. 2011;378: 815–25. doi:10.1016/S0140-6736(11)60814-3
3. Neff GW, Duncan CW, Schiff ER. The current economic burden of cirrhosis. Gastroenterol Hepatol (N Y). Millenium Medical Publishing; 2011;7: 661–71. Available: http://www.ncbi.nlm.nih.gov/pubmed/22298959
4. Nguyen GC, Tuskey A, Dassopoulos T, Harris ML, Brant SR. Rising hospitalization rates for inflammatory bowel disease in the United States between 1998 and 2004#. Inflamm Bowel Dis. 2007;13: 1529–1535. doi:10.1002/ibd.20250
5. Floyd DN, Langham S, Séverac HC, Levesque BG. The Economic and Quality-of-Life Burden of Crohn's Disease in Europe and the United States, 2000 to 2013: A Systematic Review. Dig Dis Sci. 2015;60: 299–312. doi:10.1007/s10620-014-3368-z
6. Seidell JC, Halberstadt J. The Global Burden of Obesity and the Challenges of Prevention. Ann Nutr Metab. 2015;66: 7–12. doi:10.1159/000375143
7. Buchwald H, Avidor Y, Braunwald E, Jensen MD, Pories W, Fahrbach K, et al. Bariatric Surgery. JAMA. 2004;292: 1724. doi:10.1001/jama.292.14.1724
8. Mokdad AA, Lopez AD, Shahraz S, Lozano R, Mokdad AH, Stanaway J, et al. Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. BMC Med. 2014;12: 145. doi:10.1186/s12916-014-0145-y
9. Schuppan D, Afdhal NH, Schaffner H, Popper H, Desmet V, Roskams T, et al. Liver cirrhosis. Lancet. Elsevier; 2008;371: 838–851. doi:10.1016/S0140-6736(08)60383-9
10. Byass P. The global burden of liver disease: a challenge for methods and for public health. BMC Med. BioMed Central; 2014;12: 159. doi:10.1186/s12916-014-0159-5
11. Kaplan GG. The global burden of IBD: from 2015 to 2025. Nat Rev Gastroenterol Hepatol. 2015;12: 720–7. doi:10.1038/nrgastro.2015.150
12. Chouraki V, Savoye G, Dauchet L, Vernier-Massouille G, Dupas J-L, Merle V, et al. The changing pattern of Crohn's disease incidence in northern France: a continuing increase in the 10- to 19-year-old age bracket (1988-2007). Aliment Pharmacol Ther. 2011;33: 1133–42. doi:10.1111/j.1365-2036.2011.04628.x
13. Antunes O, Filippi J, Hébuterne X, Peyrin-Biroulet L. Treatment algorithms in Crohn's – Up, down or something else? Best Pract Res Clin Gastroenterol. 2014;28: 473–483. doi:10.1016/j.bpg.2014.05.001
14. Mosca A, Leclerc M, Hugot JP. Gut Microbiota Diversity and Human Diseases: Should We Reintroduce Key Predators in Our Ecosystem? Front Microbiol. Frontiers Media SA; 2016;7: 455. doi:10.3389/fmicb.2016.00455
15. Gisbert JP, Panés J. Loss of Response and Requirement of Infliximab Dose Intensification in Crohn's Disease: A Review. Am J Gastroenterol. 2009;104: 760–767. doi:10.1038/ajg.2008.88
16. Gałecka M, Szachta P, Bartnicka A, Łykowska-Szuber L, Eder P, Schwiertz A. Faecalibacterium prausnitzii and Crohn's disease - is there any connection? Polish J Microbiol. 2013;62: 91–5. Available: http://www.ncbi.nlm.nih.gov/pubmed/23829084
17. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux J-J, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A. 2008;105: 16731–6. doi:10.1073/pnas.0804812105
18. Bodelier AGL, Smolinska A, Baranska A, Dallinga JW, Mujagic Z, Vanhees K, et al. Volatile Organic Compounds in Exhaled Air as Novel Marker for Disease Activity in Crohn's Disease: A Metabolomic Approach. Inflamm Bowel Dis. 2015;21: 1776–85. doi:10.1097/MIB.0000000000000436
19. Baranska A, Mujagic Z, Smolinska A, Dallinga JW, Jonkers DMAE, Tigchelaar EF, et al. Volatile organic compounds in breath as markers for irritable bowel syndrome: a metabolomic approach. Aliment Pharmacol Ther. 2016;44: 45–56. doi:10.1111/apt.13654
20. Pijls KE, Smolinska A, Jonkers DMAE, Dallinga JW, Masclee AAM, Koek GH, et al. A profile of volatile organic compounds in exhaled air as a potential non-invasive biomarker for liver cirrhosis. Sci Rep. Nature Publishing Group; 2016;6: 19903. doi:10.1038/srep19903
21. Krul C, Luiten-Schuite A, Baandagger R, Verhagen H, Mohn G, Feron V, et al. Application of a dynamic in vitro gastrointestinal tract model to study the availability of food mutagens, using heterocyclic aromatic

amines as model compounds. Food Chem Toxicol. 2000;38: 783–92. Available: http://www.ncbi.nlm.nih.gov/pubmed/10930699

22. Kortman GAM, Dutilh BE, Maathuis AJH, Engelke UF, Boekhorst J, Keegan KP, et al. Microbial Metabolism Shifts Towards an Adverse Profile with Supplementary Iron in the TIM-2 In vitro Model of the Human Colon. Front Microbiol. 2016;6: 1481. doi:10.3389/fmicb.2015.01481